^{99m}Tc-Hydrazinonicotinamide-annexin V
^{99m}Tc-HYNIC-annexin V

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Chemical name:	^{99m}Tc-Hydrazinonicotinamide-annexin V	
Abbreviated name:	^{99m}Tc-HYNIC-annexin V	
Synonym:		
Backbone:	Protein	
Target:	Phosphatidylserine	
Mechanism:	Binding	
Method of detection:	SPECT, planar	
Source of signal:	^{99m}Tc	
Activation:	No	
***In vitro* studies:**	Yes	
Rodent studies:	Yes	
Other non-primate mammal studies:	No	
Non-human primate studies:	No	
Human studies:	Yes	Click on protein [http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&val=468888], nucleotide [http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=4809273] (RefSeq), and gene for more information about annexin V.

Background

[PubMed]

Apoptosis, or programmed cell death, plays an important role in the pathophysiology of many diseases, such as cancer, neurodegenerative disorders, vascular disorders, and chronic hepatitis, as well as in the biology of normal cells, such as epithelial cells and immune cells (1). Apoptosis is gene regulated (2) and involves the proteolysis of intracellular components by activation of a series of proteolytic enzymes, called caspases, and changes of plasma membrane structure by translocase, floppase, and scramblase (3-5). As a result, there is rapid redistribution of phosphatidylserine (PS) from the inner membrane leaflet to the outer membrane leaflet of apoptotic cells, exposing the anionic head group of PS. On the other hand, PS is also accessible for annexin V binding in necrotic cells because of disruption of the plasma membrane.

Annexin V is a 36-kDa endogenous human protein that is produced by the epithelial cells of many tissues, such as placenta, umbilical vessels, liver, spleen, kidney, heart, uterus, and skeletal muscle, as well as by erythrocytes, leukocytes, endothelial cells, and platelets (6). Annexin V binds to PS with high affinity (K_d = 7 nM) with 8 annexin V molecules per PS molecule (3, 7, 8). Apoptosis

can be induced by chemicals, radiation, cytokines, hormones, and various pathologic conditions (5); therefore, the ability to monitor apoptosis in association with disease progression or regression should provide important information for clinical applications. Annexin V has been radiolabeled with ^{123}I, ^{125}I, and ^{99m}Tc for single-photon emission computed tomography (SPECT) imaging (9, 10), and hydrazinonicotinamide (HYNIC) has been conjugated to human recombinant annexin V to form HYNIC-annexin V (11). ^{99m}Tc-HYNIC-annexin V is being developed as an imaging agent to study apoptotic and necrotic cells in humans.

Synthesis

[PubMed]

In the method described by Blankenberg et al. (11), HYNIC was conjugated to human recombinant annexin V to form HYNIC-annexin V by use of succinimidyl 6-HYNIC. HYNIC-annexin V was purified by dialysis with a yield of >94%, and the ratio of HYNIC to annexin V was 0.9:1. A mixture of HYNIC-annexin V (100 μg) and [^{99m}Tc]pertechnetate (148-740 MBq (4-20 mCi)) was reacted in a solution containing tricine/$SnCl_2$ at room temperature for 60 min. ^{99m}Tc-HYNIC-annexin V was purified by column chromatography to give a yield of 56%, with a radiochemical purity >97% and a specific activity >1.95 MBq/μg of protein (50 μCi/μg of protein). Verbeke et al. (12) reported optimized preparation of ^{99m}Tc-HYNIC-annexin V with 95% labeling yields by reaction of 60-90 μg of HYNIC-annexin V, 0.37-1.11 GBq (10-30 mCi) of $^{99m}TcO_4^-$, 10-20 μg of $SnCl_2 \cdot 2\ H_2O$, and 1.5 mg of tricine.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

^{99m}Tc-HYNIC-annexin V has been shown to selectively bind to apoptotic Jurkat T cells (induced by serum deprivation, doxorubicin, and anti-FAS antibody) and thymocytes (induced by dexamethasone) (13). The increases in ^{99m}Tc-HYNIC-annexin V radioactivity were correlated with fluorescein isothiocyanate (FITC)-annexin V fluorescence intensities as measured by flow cytometry. The IC_{50} values of annexin V, HYNIC-annexin V, and decayed ^{99m}Tc-HYNIC-annexin V for inhibition of FITC-annexin V binding to PS were 8, 10.5, and 12.3 nM, respectively. There thus was only a minimal loss of binding affinity by the modified annexin V.

Animal Studies

Rodents

[PubMed]

Blankenberg et al. (11, 13) performed biodistribution studies in normal mice. Their results indicated high accumulation of radioactivity in the kidney (187% of injected dose (ID)/g of tissue), followed by the spleen (17% ID/g), liver (15% ID/g), stomach (5% ID/g), and lung (3% ID/g) at 60 min after injection of 0.74-1.85 MBq (0.02-0.05 mCi) of ^{99m}Tc-HYNIC-annexin V. The radioactivity

levels in the brain, heart, and thymus were the lowest (<0.2% ID/g), and the radioactivity in the blood was 2%. There was a 3-fold increase in hepatic accumulation of ^{99m}Tc-HYNIC-annexin V in mice treated with anti-FAS antibody to induce apoptosis in the liver compared with control mice. Accumulation of ^{99m}Tc-human serum albumin in both the kidney and liver was similar in the FAS-treated and control mice. There was also a 2- to 6-fold increase in ^{99m}Tc-HYNIC-annexin V accumulation at apoptotic sites in mice bearing 38C13 B-cell lymphomas treated with cyclophosphamide and in rats with transplanted heterotopic cardiac allografts.

Blankenberg et al. (14) performed a ^{99m}Tc-HYNIC-annexin V SPECT imaging study in cyclophosphamide-treated rats (8-10 weeks old); their results showed increased accumulation of radioactivity in the femur, pelvis, vertebrae, and spleen as early as 8 h after cyclophosphamide treatment (100 mg/kg). Older rats (5-6 months old) showed a slower response to cyclophosphamide treatment and delayed recovery of bone marrow and splenic tissues. Therefore, the immunosuppressive effect of cyclophosphamide can be visualized by SPECT imaging with ^{99m}Tc-HYNIC-annexin V.

Using ^{99m}Tc-HYNIC-annexin V SPECT, Takei et al. (15) studied the effects of chemotherapy with gemcitabine and cyclophosphamide in rats bearing allogenic hepatomas. After chemotherapy, the accumulation of ^{99m}Tc-HYNIC-annexin V in the tumors increased significantly in the gemcitabine group (0.062% ID/g × kg) and in the cyclophosphamide group (0.050% ID/g × kg) compared with that in the control group (0.031% ID/g × kg; $P < 0.01$). In contrast, the uptake of 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG [http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=micad.chapter.FDG]) in the tumors decreased significantly in the gemcitabine and cyclophosphamide groups compared with the control group ($P < 0.01$). In addition, tumor uptake of [^{18}F]FDG negatively correlated with ^{99m}Tc-HYNIC-annexin V accumulation ($r = -0.75$; $P < 0.01$). In the gemcitabine and cyclophosphamide groups, the percentages of terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL)-positive cells were significantly higher ($P < 0.01$) than in the control group (10.2 ± 1.7, 8.0 ± 1.5, and 5.2 ± 1.5%, respectively), whereas glucose transporter-1 expression showed little change in histologic analyses. Hence, these data indicate that an enhanced apoptotic reaction correlated with suppressed tumor glucose utilization after cytotoxic chemotherapy as determined by radiotracers and histologic evaluation.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

Various SPECT studies using ^{99m}Tc-HYNIC-annexin V have been performed in patients with head-and-neck carcinoma and showed that absolute uptake values in the primary tumors correlate well with the number of apoptotic cells as measured by the TUNEL assay or other histologic analyses (16-19). However, SPECT failed to identify most of the lymph node lesions detected by computed tomography, mainly because the lymph nodes are smaller in size than the primary tumors and contain mostly focal metastases.

In a study of 33 patients with lymphoma (*n* = 26) and other tumors, Kartachova et al. (20) concluded that partial or complete tumor response to therapy was associated with a marked increase in ^{99m}Tc-HYNIC-annexin V in the tumors. In contrast, patients with stable or progressive disease showed low ^{99m}Tc-HYNIC-annexin V accumulation before treatment and no increase after treatment. Therefore, ^{99m}Tc-HYNIC-annexin V SPECT may be used to identify patients who would respond to therapies and to assess the effectiveness of therapies.

Kemerink et al. (21) reported on SPECT studies in 6 normal volunteers after injection of 250 MBq (6.8 mCi) of ^{99m}Tc-HYNIC-annexin V. The kidneys received the highest dose of radioactivity (49.7% ID) at 3 h after injection, followed by the liver (13.1% ID), the red marrow (9.2% ID), and the spleen (4.6% ID). More than 90% of the blood activity was cleared rapidly with a half-life of 24 min. The radioactivity was excreted almost exclusively through the urine (22.5% ID at 24 h), and hardly any radioactivity was seen in the feces. Absorbed doses were 196 μGy/MBq (0.73 rad/mCi) for the kidneys, 41 μGy/MBq (0.15 rad/mCi) for the spleen, 16.9 μGy/MBq (0.063 rad/mCi) for the liver, and 8.4 μGy/MBq (0.031 rad/mCi) for the red marrow. The effective dose was 11.0 ± 0.8 μSv/MBq (40.7 ± 3.0 mrem/mCi).

References

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